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## Phytochemical Screening of *Eugenia Caryophyllata* (Clove Leaves) and Characterization of Its Essential Oil

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### Abstract

Qualitative Phytochemical screening were conducted on Clove plant using four different solvents and they include; Ethyl-acetate, Distilled Water, Methanol and N-Hexane as the extracting solvents and the of presence of Alkaloid, Tannin, Saponin, Flavonoid, Phenol, Steroids, Glycoside and Terpenoid were checked on the four different extracts and the results is as follows: Ethyl-acetate and Methanol extracts showed in abundance the presence of alkaloid(+ +), while N-Hexane and Water extracts showed the presence(+). N-Hexane extracts showed in abundance the presence of Tannin (+ +), while water and Ethyl-acetate extracts showed the presence of alkaloid, and Methanol extract showed none presence of Tannin (-). Methanol and water extracts showed the presence of Saponin in abundance (+ +), while N-hexane and Ethyl-acetate showed presence the Saponin (+). Methanol showed in abundance the presence the Flavanoids (+ +), while water and Ethyl-acetate extracts showed the presence of Flavonoids and N-Haxane showed none presence (-). However, Water extract show in abundance the presence of Phenol (+ +), followed by Ethyl-acetate and Methanol extracts which showed as well the presence of Phenol (+), while N-Haxane extracts showed none presence of Phenol (-). Meanwhile, the presence of Steroids were not found in any of the extracts. Glycoside's presence were in the abundance in the extracts of Ethyl-acetate (+ +), Water and Methanol extracts (+ +), while N-Hexane showed presence of Glycoside (+). Terpenoid presence were in abundance (+ +) in Methanol extract, followed by Ethyl-acetate and water extracts which showed the presence of terpenoids(+), while N-Hexane extract showed none presence of Terpenoid. Physiochemical analysis results of Clove plant were as follows: Acid value 3.64(% as oleic acid), Saponification value 44.05 (MgKOH/g), Iodine value 9.0 (g I/100 of oil), Specific gravity 1.042, Refractive index 1.429, Free fatty acid 1.82 (% as oleic acid) Peroxide value 7.45 (Meq/Kg), Colour (Pt-Co) (Yellow) and Ester value 28.04( MgKOH/g). However, Mineral analysis results were as follows: Ca 0.600(mg/Kg), Mg 0.117(Mg/Kg), Mn 0.950(Mg/Kg), Fe 0.440(Mg/Kg), P 7.550(Mg/Kg), Na 0.001 (Mg/Kg), Zn 0.390(Mg/Kg), Cu 0.869(Mg/Kg), Pb 0.194(Mg/Kg) and K 0.039(Mg/Kg). TLC results as regards to distance traveled are as follows, Methanol, Ethyl-acetate, N-Hexane and Distilled water.

**Keywords:** Clove plant; Its Essential Oil; Analytical Characteristics.

### Introduction

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants precisely, medicinal plants which grown in different parts of the world and are known to be the richest resource of drugs of traditional systems of medicine, modern medicine, nutraceuticals, food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. However, clove plant as one of the medicinal plants found in Nigeria were evaluated in this work. Cloves are the aromatic flower and as well the dried buds of a flower tree which belongs to the plant family known as Myrtaceae, Syzgium aromaticum. They are native to the Maluka Islands in Indonesia and are commonly used as spices [2]. Clove plant tree is as well-known as *Eugenia arena*, reaching the height of 15 to 30 feet tall. It has a pale yellowish gray in colour and smooth. It has opposite ovate (egg-shaped) leaves range from 3 -6 inches long. Its flower red white, ball-shape, and grow in clusters. The flowers when gathered are at first of a reddish colour but on drying, they assume a

a deep brown cast [3]. Clove is a herb and as well an evergreen plant where various part of it especially, the dried bud, stems and leaves are used for medicinal purposes which include, protection of the liver, boosting of immune system, aids in digestion, having anti-microbial properties, fighting against cancer, and aphrodisiac properties as well [4]. Clove (*Eugenia caryophyllata*), has been used for thousands of years in the Asia continents not only for culinary purposes but also as a medicine for many ailments. Ayurvedic medicine used Clove for tooth decay, halitosis, bad breath. Meanwhile, in the ancient Chinese kingdom, during the Han dynasty (207 BC to 220 AD) those who addressed the Chinese emperor were required to hold cloves in their mouth to mask bad breath. Also, due to broad knowledge of the traditional Chinese physicians towards medicinal plants, they have long applied Cloves to treat many ailments such as ringworm, Hernia, athlete's foot and other fungal infections and as a stimulant for the nerves [5]. Clove as a plant, first arrived in Europe around the 4<sup>th</sup> Century AD as a highly coveted luxury, and this led the European doctor who once breathed through clove-filled leather beaks towards off the plague [6]. In addition, since clove became a luxury upon its arrival in Europe, a medieval German herbalist had used clove as part of anti-gout mixture; because of its availability in Europe as well, it was prized as a treatment for flatulence, nausea, vomiting and, diarrhea. It was also used for the treatment of infertility, warts and cough [7].



**Fig. 1:** Shows the pictures of Clove Plant.



**Fig 1:** Shows the pictures of Clove Plant.

## 2. Materials and Methods

### 2.1 Chemical

All chemicals used were of analytical grade made up of JHD and BDH chemical Ltd Poole England. Iodine crystal, Potassium Iodide, Ethanol, Ethyl-acetate, N-Hexane, Distilled water, Conc. Sulphuric acid, Perchloric acid, Hydrochloric acid, Copper Sulphate, Potassium tartrate, Ferric chloride, Potassium Hydroxide, Lead Acetate, Sodium hydroxide, Aluminium trichloride, Acetic anhydride and Whatmann filter paper, Chloroform, Alumina and Methanol.

### 2.2 Plant Material Collection, Identification and Pre-Treatment.

The samples of Clove [*Eugenia Caryophyllata*] were sourced from Eke-Awka Market in Anambra State, Nigeria. It was identified by a Taxonomist of the Botany Department of Nandi Azikiwe University Awka, Anambra State, Nigeria. The samples were air dried at room temperature for 72hrs and were later oven dried with vacuum drying oven [Model no 267 Pa] for 30mins at a temperature of 50<sup>o</sup> C. The dried Clove Samples were subsequently ground using Mammonlex super blender mill great [No 4 A0-0018, Screen having a mesh size of 1.0mm].

### 3. Phytochemical Analysis

20g of ground sample of Clove were weighed differently into 250ml Conical flask and were soaked with 200ml of the extracting solvents then stoppered and were allowed for 24hours, after which they were decanted and filtered adequately. Hereafter, subjected to preliminary phytochemical Screening to identify the chemical constituent of the sample in the four different Solvents extracts which include: Ethyl-Acetate, Methanol, N-Hexane and Distilled Water. Test for the presence of Alkaloid, Saponin, Tanning, Steroids, Phenol, Glycoside and Flavonoids and Terpenoids were carried out, and as well Thin Layer Chromatography on the extracts.

#### 3.1. Alkaloid Determination

To 1ml of the extract in the test-tube, add 1ml Wagner's reagent and the presence of alkaloid was confirmed by the slight reddish precipitate. [8].

#### 3.2. Glycoside Determination

To 2ml of the extract in the test-tube, add 5ml of distilled water, add 2ml of Conc. H<sub>2</sub>SO<sub>4</sub> and boil for 15minutes using water bath. Allow cooling and then neutralizing with 2ml of 20% KOH, add 1ml of equal volume of Fehling solution A and B and boil for 15minutes. The formation of brick red colouration confirmed the presence of Glycoside.

#### 3.3. Terpenoid Determination

To 2ml of the extract in the test-tube, add 2ml of acetic anhydride and 2ml of Conc H<sub>2</sub>SO<sub>4</sub>, the presence of terpenoids was confirmed by the colour change from blue to green rings. [8].

#### 3.4. Tannin Determination

To 1ml of the extract in the test-tube, add 0.5ml of 20% freshly prepared KOH. The presence of Tannin was confirmed by the formation of dirty white Precipitate [9].

### 3.5. Saponin Determination.

To 3ml of the extract in the test-tube, add 2ml of equal volume of feeling solution A and B, then boil for 5minutes. The presence of Saponin was confirmed by the formation brick reddish precipitate.

### 3.6. Flavonoids Dertermination

To 1ml of the extract in the test-tube, add 2 drops of Alcohol and 0.5ml of Conc.  $H_2SO_4$ , the poresence of Flavanoid were confirmed by the formation of yellowish colouration. [8].

### 3.7. Phenol Determination

To 1ml of the of extract in the test-tube, add 1ml of distilled water and 2-3drops of 5% NaOH, and the presence of Phenol were confirmed by the colour change from yellow to bright orange.

### 3.8. Steroid Determination

To 1ml of the extract in the test-tube, add 1ml of acetic anhydride and 2ml of Conc.  $H_2SO_4$ ; the presence of steroid were confirmed by the colour change from blue to green [8].

## 4. Oil Extraction and Characterization

### 4.1. Oil Extraction

Steam distillation was employed for the extraction of the essential oil from the Clove plant. However, when passed steam generated by the steam pot to the plant material, tiny pockets holding the essential oil opens to release the essential oil molecules without doing any damage to these delicate components, and the obtained distillate will contain a mixture of water vapour and essential oil which finally return to their liquid form in the condensing apparatus and later separated either with separating funnel or Florentine separator.

### 4.2. Characterisation

Physiochemical analysis of Clove oil was conducted and parameters are: Density and refractive index which were determined using the method adopted by [10]. Peroxide value and Saponification value were carried out according to various standards of [11]. Acid value, ester value, frees fatty acid value were determined according to [12], Iodine value according to Wij's method as described by [13]. Colour was determined using a lovibond tonometer [14].

## 5. Thin Layer Chromatography (TLC)

### 5.1. Preparation of Chromaplate

The glass plates were cleaned and dried using air drying oven at a temperature of  $50^{\circ}C$  for 15minutes. Slurry was prepared by mixing Alumina with distilled water and about 15% of Calcium Sulphate were incorporated into the slurry which serves as a binder and binds the Alumina on the glass plate were prepared in 250ml cleaned beaker with a continuous stirring until the homogeneous mixture of the additives is achieved. The slurry was coated on the plate through slide and after which they were air dried under room temperature for 30minutes and the chromaplates were activated by heating in heating in the vacuum oven at temperature range of  $100 - 110^{\circ}C$  For 30minutes.

### 5.2. Loading of the sample

The plates were allowed to cool after heating for

30minutes. Meanwhile, about 2cm were marked from the botton as the Origin. The working suspension (The extracts) was loaded at the centre of the slide and about 2cm above from the edge.

### 5.3. Development of the Chromatogram

The TLC development tank was saturated with Chloroform and Ethanol for the analysis of the plant extracts. However, the coated chromatogram plates were left in the TLC tank without touching the baseline by the solvent and left for development.

### 5.4. Spot Visualization

About 0.5g of Iodine crystals were weighed and kept in the TLC tank and covered with glass plate to saturate the tank with the vapour. However, the plates were then left in the iodine 3hours after which it was later removed and the readings were taking.

## 6. Mineral Determination

0.2g of the grand sample of Clove was digested using Hydrochloric acid and Perchloric acid in the ratio of 2:1. The mixture was heated at  $100^{\circ}C - 105^{\circ}C$  for 1hour 45minutes using a hot plate. The digested sample was making up to 250ml marked in the volumetric flask with distilled water. The aliquot solution was checked for their mineral contents using Atomic Absorption Spectrophotometer (A.A.S.) with model no VGP 210 Buck.

## 7. Results and Discussion

### Result

**Table 1:** Qualitative phytochemical screening of *Eugenia Caryophyllata*

| Paramters  | Ethyl-acetate | Methanol | N-Hexane | Water |
|------------|---------------|----------|----------|-------|
| Alkaloid   | ++            | ++       | +        | +     |
| Tannin     | +             | -        | ++       | +     |
| Saponin    | +             | ++       | +        | ++    |
| Flavonoid  | +             | ++       | -        | +     |
| Phenol     | +             | +        | -        | ++    |
| Steroid    | -             | -        | -        | -     |
| Glycoside  | ++            | ++       | +        | ++    |
| Terpenoids | +             | ++       | -        | +     |

+ Shows the presence of phytochemical, ++ Shows the presence of phytochemical in abundance; - Shows the absence of phytochemical constituent.

### 7.2. Physiochemical Properties

**Table 2:** Physiochemical properties of *Eugenia Caryophyllata*.

| Parameters                         | <i>Eugenia Caryophyllata</i> | <i>Olibanum (STD)</i> |
|------------------------------------|------------------------------|-----------------------|
| Acid Value(% as oleic acid)        | 3.64                         | 3.28                  |
| Saponification Value(MgKOH/g)      | 44.05                        | 33.23                 |
| Iodine Value(gI/100g of Oil)       | 9.00                         | 5.40                  |
| Specific gravity                   | 1.042                        | 0.900                 |
| Refractive Index $n_{20}^{\circ}C$ | 1.429                        | 1.443                 |
| Free Fatty acid(% as oleic acid)   | 1.82                         | 1.64                  |
| Peroxide Value(Meq/Kg)             | 7.45                         | 5.80                  |
| Colour Value Pt-Co                 | Yellow                       | Yellow                |
| Ester Value(MgKOH/g)               | 28.04                        | 9.50                  |

Values are means and also analyzed in triplicate.

### 7. 3. Mineral Constituent of *Eugenia Caryophyllata*

**Table.3:** Mineral Constituent of *Eugenia Caryophyllata* (Clove)

|    | <b><i>Eugenia Caryophyllata</i></b> | <b>(WHO. STDs for plants in Mg/kg)</b> |
|----|-------------------------------------|--|
| Ca | 0.600                               | 0.1-1.5                                |
| Mg | 0.117                               | 0.1-1.0                                |
| Mn | 0.950                               | 50.00                                  |
| Fe | 0.440                               | 20.00                                  |
| P  | 7.550                               | 1.0-22.00                              |
| Na | 0.001                               | 1.0-6.00                               |
| Zn | 0.390                               | 50.00                                  |
| Cu | 0.869                               | 10.00                                  |
| Pb | 0.194                               | 2.00                                   |
| K  | 0.039                               | 0.0003-0.0008                          |

Values are means and also analyzed in triplicate.

#### Discussions

The present study carried out on the various extracts of Clove leave (*Eugenia Caryophyllata*) revealed that there are presence of medicinal active constituents. Meanwhile, the medicinal beneficial effects of plants typically results from the secondary products present in the plant, it is usually not attributed to a single compound but a combination of the metabolites [15]. The phytochemical active compounds of Clove leave were qualitatively analyzed separately and the results are presented in the Table 1. In the screening process, Alkaloid, Tannin, Saponin, Flavonoid, Phenol, Steroid, Glycoside and Terpenoid showed various degrees of results in different solvents medium. However, the medicinal value of plants lies in some chemical substances that have definite physiochemical action on the humanbody [16]. Difference phytochemicals has been found to possess a wide range of activities, which may help to protects against chronic diseases such as heart diseases and stroke, saponins protects against hypercholesterolemia and antibiotics properties [16]. Steroids and tepernoids shows the analgesic for central nervous system activity. The importance of Alkaloids, Saponins and Tannins in various antibiotics used in treating common pathogenic strains has recently been reported by [17, 18].

However, according to [19, 20], a glycoside appeared to be the major bioactive component that offers antisecretory and antiulcer effects. Meanwhile, looking at the physiochemical analysis of clove oil conducted, which include Acid value, Peroxide value, Saponification value, Specific gravity, Refractive index, Free fatty acid, Iodine value and Clour as shown in the Table 2 which were all done according to [10, 11, 14, 21]. However, when compared with some other medicinal plant species especially *Olibanum* oil which has similar medicinal properties related to Clove which were used as a standard to clove oil in this work, it was observed that the physiochemical properties of Clove oil were found to be greater than that of *Olibanum* oil [22, 23]. However, Clove oil is known to contain several compounds but it's major compound known to contain is Eugenol up to about 85-90% which is the source of its antifungal, anesthetics and antiseptic properties and as well as Eugenol Acetate but at the percentage of 9-10%. Meanwhile, Clove plant were found to contain some mineral elements which aids in body building and maintenance for good healthy living and they include: Ca, Mg, Fe, Na, Pb, Zn, P, K, Cu, and Mn. However, some of these minerals are pertinent to the body but in various degrees or amount, Calcium and Phosphorus

are of great importance to the body compared with other minerals, and their functions include: strong teeth, strong bone and skeletal building but calcium are needed in the body at a higher percentage than Phosphorus. In addition, the higher or lower level of Phosphorus in the body could cause some medical complication such as heart diseases and joint pain. Manganese plays a vital role in the body physiological process as a constituent of multiple of enzymes and activator of other enzymes. Zinc is essential trace element because very small amount of it are necessary for human health and as well serves as an immune booster for treating; common cold, recurrent ear infection and preventing lower respiracy. Potassium and Sodium have similar characteristics, in terms of regulating the body system and balancing. In addition, Potassium is of importance to the body for both cellular and electrical function and as well regulates the water balance and acid-base balance in the blood tissue, While Sodium contributes to the proper muscle-contraction and nerve impulse conduction. Magnesium is a confactor in over 300 enzymes system that regulates diverse biochemical reactions in the body including protein synthesis, blood glucose control, blood pressure regulation, glycolysis and as well the fourth must abundant mineral in the body. However, the presence of Copper is important for it aids normal body metabolism and it is as well a component of hemocyanin. Finally, Iron is a very vital mineral element because it helps in the growth and also carries oxygen around the body. In addition, one important sign of lack or low iron in the body is tiredness and fatigue. Thin Layer Chromatography were conducted on the four extracts of the Clove plant leave which include Ethyl-acetate, Methanol, Distilled Water and N-Hexane using Chloroform and Ethanol as the eluting solvent in a ratio of 9:1. Meanwhile, observations based on the distances traveled by the sample solutes and the solvent were seen and their degrees of distance travels were as follows: Methanol has the greatest distance, followed by Ethyl-acetate, N-Hexane and Distilled Water.

#### 9. Clove and Its Therapeutics Uses

Clove as a plant is of great importance especially when considered the entire parts of it which include, Bud, Leaves, Roots and it's Oil in terms of their medicinal values and as well as their Therapeutic uses are enormous to mention but few. However, Clove could be used for the treatment of Cough, Dizziness, Epilepsy, as a sleep inducer, Blood thinner and Mental Stimulant [24]. In addition, Clove is the active ingredient in several mouthwashes used in preparing toothache, pain-relief, it assist the stimulation of blood circulation in the body, making it very useful for people who have cold extremities, it fights against germs, viruses and bacteria; it encourages the loosening of phlegm from the respiratory system and as well as promoting sweating with fevers, and flu which is very healing [25]. Also, a few drops of Clove oil in water will stop vomiting, and it's tea taking will help to stop diarrhea, intestinal spasms and nausea, and as well as gas ; it also helps in prevention breakdown of the retina eye, which slows down macular degeneration and aids vision in old age. According to [25], Sniffing the spicy aroma of Clove reduces headaches, irritability, drowsiness and as well helps to increase memory recall and he also states that many foods and substances contains antioxidants which absorb free radicals (Toxins). In addition, that scientist at Tufts



University has developed a scale for the U.S. Department of Agriculture called the ORAC (Oxygen Radical Absorption Capacity) test. The test states that the higher ORAC score is, the more capable that particular food, spice or herb is of destroying free radicals (antioxidants). Meanwhile, according to essential oils Desk 2<sup>nd</sup> edition, which states that essential oils have the highest ORAC scores of any known substance. Here are some essential oil and their ORAC scores: Eucalyptus (*Eucalyptus globules*) 24, 1157, Cinnamon Bark (*Cinnamamum verum*) 103,44, Lemon grass (*Cymbopogen flexnosus*) 17,765, Oregano (*Origanum compactum*) 153,007, Thyme (*Thymus vulgaris*) 159,590, Clove (*Syzygium aromaticum*) 10,786,875.

## Conclusion

The importance of Clove plant can never be over emphasized. However, Clove oil is not ideal for pregnant or nursing mothers, children between the age of 2 years and below rather, older children and people over 65 years because, it aids in increasing strength if necessary. Moreover, Clove is the Champion of all the essential oil with an ORAC score over 10 million! That means a drop of Clove oil contains 400x more antioxidants per units volume than wolf berries, the most powerful of all known fruits, and a 15ml bottle of Clove oil has the antioxidant capacity of 40 quarts of blue berries [25]

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